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ORIGINAL PAPER

Evaluation of elemental allelopathy in *Acroptilon repens* (L.) DC. (Russian Knapweed)

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Abstract Although *Acroptilon repens* (L.) DC. (Russian knapweed) is known to concentrate zinc (Zn) in upper soil layers, the question of whether the elevated Zn has an allelopathic effect on restoration species has not been addressed. Experiments were conducted to investigate whether soils collected from within infestations of *A. repens* (high-Zn) inhibit the germination or growth and development of desirable restoration species, compared to soils collected adjacent to an *A. repens* infestation (low-Zn). Four bioassay species [*Sporobolus airoides* (Torrey) Torrey (alkali sacaton), *Pseudoroegneria spicata* (Pursh) A. Love (bluebunch wheatgrass), *Psathyrostachys juncea* (Fischer) Nevski (Russian wildrye) and *A. repens*] were germinated in a growth chamber and grown in a greenhouse in both soils and received treatments for the alleviation of Zn toxicity (P, Fe, Fe-oxide, and soil mixing) to isolate the effects of elevated soil Zn on plant performance. Percent germination, total plant biomass, tiller and stem number, inflorescence number, and tissue metal levels were compared among soil types and treatments for each species. There was no evidence from any of the indicators measured that high-Zn soils reduced plant performance, compared to low-Zn soils. Tissue Zn levels barely approached the lower range of phytotoxic levels established for native grasses. Older plants with longer exposure times may accumulate higher Zn concentrations. *S. airoides* and *A. repens* both had higher biomass in the high-Zn soil, most likely due to increased macronutrient (N and P) availability. As the Zn levels in the soils used in this study were much higher than any levels previously reported in soils associated with *A. repens*, it is unlikely that the elevation of soil Zn by *A. repens* will hinder germination or growth and development of desirable grasses during establishment.

Keywords Iron · Phosphorus · Plant interference · Plant-soil feedback · Restoration · Weed invasion · Zinc toxicity

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Introduction

Acroptilon repens (L.) DC. (Russian knapweed) is a perennial forb native to Eurasia, which has been designated as a noxious weed throughout much of Western North America. It establishes dense monocultures with up to 100 shoots m^{-2} (Selleck 1964), crowding out other vegetation. The persistence of these monocultures has been attributed to a number of factors, including adaptation to disturbance, an extensive root system, and interference with neighboring plants through the production of organic allelopathic chemicals (Selleck 1964; Goslee et al. 2001; Grant et al. 2003). It has also been suggested that *A. repens* may interfere with neighboring plants by concentrating zinc (Zn) in the top soil layers through the accumulation of high Zn levels in stems and leaves, which are then released into soils with litter deposition and decomposition. At a site in Wyoming, USA, the Zn levels found in tissues of *A. repens* were 32 mg kg^{-1}, compared to Zn levels of 6 mg kg^{-1} found in native grasses (Bottoms 2001). The top 7.5 cm of soils within the *A. repens* infestation were found to contain 1.6 mg kg^{-1} of bioavailable Zn compared to 0.11 mg kg^{-1} of Zn in soils associated with native grasses. Similar results were found in another study at two additional sites in Wyoming, USA, where increases from 0.61 to 6.6 mg kg^{-1} and 0.062 to 0.146 mg kg^{-1} of bioavailable Zn were found in soils collected from within *A. repens* infestations, compared to adjacent vegetation (Tyrer 2005). Bottoms (2001) estimated that it would take as long as 200 years for Zn levels to return to normal levels after eradication of *A. repens* due to low mobility of Zn in soils, and that the persistence of Zn may hinder restoration by inhibiting establishment of desirable species.

In general, interference between plants has been discussed in terms of the production of toxic organic chemicals (Harper 1977; Callaway and Aschehoug 2000; Bais et al. 2003). Interference mediated through the accumulation and concentration of inorganic elements, known as ‘‘elemental allelopathy’’ (Boyd and Martens 1998), has received less attention. In general, the study of biochemical allelopathy has been characterized by speculation and assumptions (Inderjit and Del Moral 1997). To help prevent this, Fuerst and Putnam (1983) proposed the following criteria for determining the presence of biochemical allelopathy: the identification of symptoms, isolation and characterization of the toxin, identification of levels found in nature, and the release, movement and uptake of the toxin. These same criteria should be applied to the study of elemental allelopathy, in order to avoid the problems that have characterized the study of biochemical allelopathy.

To test whether the elevation of Zn in soils associated with infestations of *A. repens* negatively affects desirable vegetation, treatments can be applied to plants and soils to prevent or alleviate Zn toxicity. Because Zn and iron (Fe) are both divalent and have similar radii, excessive Zn availability can result in substitution of Zn for Fe in critical processes, resulting in Fe deficiency. Therefore, supplementing Fe uptake has been found to reduce stress caused by high Zn (Kaya and Higgs 2001). Although the mechanism is not entirely understood, high amounts of phosphorus (P) have been found to reduce Zn activity in plant tissue, even to the point of inducing Zn deficiency (Singh et al. 1986). Iron oxides, when applied to the soil, have been shown to reduce uptake of all metals by immobilizing them in the soil (Lombi et al. 2002). Plowing or disking sites where *A. repens* has been eradicated may help to dilute the concentration of Zn at the soil surface; however, this technique has not been researched.

Of the criteria set by Fuerst and Putnam (1983) for the determination of allelopathy, the isolation and characterization of the toxin, identification of levels found in nature, and the release, movement and uptake of the toxin are somewhat easier to study when working with individual elements compared to complex biochemicals. Therefore, this study focuses on identifying the symptoms of toxicity, which is the criterion largely ignored by previous studies of elemental allelopathy. Our objective was to test whether the accumulation of Zn in soils associated with infestations of *A. repens* inhibits the germination or growth and development of desirable grasses, by applying treatments that alleviate or prevent Zn toxicity to plants grown in soils with elevated Zn levels.

Methods

Two experiments were implemented: a germination experiment and a growth and development experiment. Aspects that are common to both experiments are described first and then unique aspects for each experiment are described afterwards in detail.

Bioassay species selection

The four plant species used as bioassays in both experiments were: *Sporobolus airoides* (Torrey) Torrey (alkali sacaton), *Pseudoroegneria spicata* (Pursh) A. Love (bluebunch wheatgrass), *Psathyrostachys juncea* (Fischer) Nevski (Russian wildrye), and *A. repens*. All three grass species were purchased commercially (Granite Seed Co., Lehi, UT, USA). *A. repens* seed was collected from the same site in Salt Lake County, UT, USA where soils were collected (see below) and sorted to remove hollow, nonviable seeds. *P. spicata* is a cool-season bunchgrass and is a poor competitor with many invasive weeds (Monsen et al. 2004). It was speculated that this poor competitive ability might correlate to intolerance of interference, such as elevated Zn, and provide a sensitive bioassay. *S. airoides* is a sod-forming, warm-season grass, and an important restoration species (Monsen et al. 2004). *P. juncea* is a cool-season bunchgrass that has been shown to do well in soils that have been previously inhabited by *A. repens* and was expected to be tolerant of elevated Zn levels (Bottoms and Whitson 1998; Monsen et al. 2004). *A. repens* was included to assess autotoxicity, and to determine the effects of Zn-ameliorative treatments for management considerations.

Soils

Soils were collected at a site in Salt Lake County, UT, USA near the Salt Lake City International Airport (40° 47′46″N, 111° 56′57″W). The site was used for livestock grazing at one time, but in the recent past has remained vacant while the area around has been developed for industrial use (pers. obs.). Vegetation mainly consisted of noxious weeds and other nonnative species, including *Bromus tectorum* L. (cheatgrass), *Cardaria draba* (L.) Desv. (hoary cress), *Aegilops cylindrica* Host (jointed goatgrass), *Melilotus officianalis* (L.) Pall. (yellow sweetclover), and *A. repens*. The site is considered to have the worst *A. repens* infestation in Utah (pers. obs.).

Soil with elevated Zn levels (high-Zn soil) was collected from the top 7.5 cm layer within an *A. repens* monoculture (Bottoms 2001). Soil without elevated Zn levels (low-Zn soil) was collected from the top 7.5 cm layer in an *A. repens*-free area approximately 30 m from where the high-Zn soil was collected. Both soils were classified as fine-silty, mixed, mesic Typic Calciaquolls (USDA 1974). Soil samples were analyzed for selected chemical and physical properties at the Utah State University Soil Analysis Laboratory (USUAL 2005) (Table 1). Nitrogen (N) content is expressed as total N. P and potassium (K) were extracted with $NaHCO_3$. Zn content was determined using a diethylenetriaminepentaacetate (DTPA) extraction. These and all other soil analysis methods follow Gavlak et al. (2003). Both soils were stored inside a nonheated building for ~3 months prior to use.

Ameliorative treatments

For both experiments, treatments were added to both low and high Zn soils for comparative purposes. This was important for the P and Fe treatments, to detect whether differences were caused by amelioration of Zn stress or just the addition of nutrient elements. Each experiment also had a low and high Zn control, where no ameliorative treatments were added. Both experiments received a low and high P treatment (150 and 400 mg kg^{-1}, respectively), as powdered calcium phosphate monobasic $Ca(H_2PO_4)_2$ (Aldrich Chemical Co., Milwaukee, WI, USA). Both experiments received a high and low Fe treatment.

Table 1 Properties of soils used in germination and growth and development experiments collected from within (High-Zn) and adjacent to (Low-Zn) an *A. repens* infestation, in Salt Lake County, UT, USA

Soil type	Texture	pH	Salinity (dS/m)	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Zn (mg kg^{-1})
Low-Zn	Clay loam	7.8	0.7	0.74	28.4	530	8.6
High-Zn	Loam	7.6	0.8	1.02	52.9	350	15.1

For the germination experiment, Fe was mixed with soil as iron sulfate ($FeSO_4$, Hi-Yield Iron Plus, Bonham, TX, USA), at low (150 mg kg^{-1}) and high (400 mg kg^{-1}) rates. These rates are based on the total amount of Fe added, and do not include other ingredients, such as sulfur (S), N or inert ingredients. For the growth and development experiment, Fe was applied foliarly as 10 ml of ferricethylenediaminedi(o-hydroxyphenylacetate) (FeEDDHA) (GrowMore Iron EDDHA Chelate 6%, Gardena, CA, USA), mixed with a non-ionic surfactant (Hi-Yield Spreader/Sticker, Bonham, TX, USA) on day 35 and again on day 55. The low treatment was at 0.1 M Fe and the high treatment was at 0.5 M Fe. The germination experiment received a Fe-oxide (Fe_2O_3, Ferric Oxide Powder, J.T.Baker, Philipsburg, NJ, USA) treatment at low and high levels (4 and 10%, by weight, respectively); however, this treatment was cost-prohibitive to apply to the growth and development experiment. Both experiments also had a mixed treatment, where the high-Zn soil was diluted at a ratio of 1:1 with low-Zn soil. This was intended to simulate the effects of mixing the soil through plowing or disking an infestation in the field.

Germination experiment

This experiment was a complete factorial arranged in a completely randomized design with treatments, soil type, and species as the three factors. Soils were sieved through a 1.5×1.5 mm^2-mesh screen and placed (2 cm deep) into square clear plastic germination boxes with dimensions of $11 \times 11 \times 3.5$ cm^2 (Hoffman Manufacturing, Albany, OR, USA). Treatments were incorporated homogeneously into the soils before placement. Each germination box received 25 seeds of one species, placed 1 cm below the soil surface. Each species was assigned randomly to a germination box until each soil type/treatment/species combination was replicated five times. Germination boxes were randomly placed in a growth chamber (Model I-35LLVL, Percival Scientific, Perry, IA, USA) that was set with a 12 h photoperiod, and day/night temperatures of 25/10 °C (Copeland 1978). Germination boxes were rotated every 2 days within the germination chamber to account for environmental variation. Each germination box was initially watered with 100 ml of deionized water and then maintained at field capacity. A fungicide (Hi-Yield Captan Fungicide 50% WP, Bonham, TX, USA) was applied to all germination boxes and supplemented as needed. Germination was defined as a cotyledon or coleoptile emerging 1 cm from the soil surface, and was recorded over the course of 50 days. After the completion of the experiment, three randomly selected high-Zn soil samples from each treatment and three samples from both the mixed treatment and the low-Zn soil control were analyzed for bioavailable Zn and Fe at the USU Soil Analysis Laboratory, using a DTPA extraction and an inductively-coupled plasma spectrophotometer (USUAL 2005). It was assumed that the treatments applied to the high-Zn soils from the germination experiment would have similar effects on the low-Zn soils, relative to their control, as well as the soils used in the pot study.

Growth and development experiment

This experiment was a complete factorial arranged in a completely randomized design with factors consisting of soil type, treatment and species. Eight-liter pots were filled with a bottom layer of 2.7 kg of low-Zn soil and a top layer of 1 kg of soil, that was either low-Zn, high-Zn, or a 1:1 mixture of the two for the mixed treatment. All ameliorative treatments, except for the foliarly applied Fe, were homogeneously incorporated into the top layer of soil before it was added to the pots. One 8-week-old seedling was transplanted randomly into each pot, until each species/treatment combination was replicated five times. Transplanted seedlings were grown in $4 \times 4 \times 6$ cm^3 plugs of low-Zn soil, which was transplanted with the seedlings to avoid root damage. Plants were grown in a greenhouse that was maintained between 17 and 26 °C with evaporative coolers. A shade cloth (Sundance Supply Co., Olga, WA, USA) was used to reduce ambient solar radiation in the greenhouse by 47%. The experiment began on 17 June 2004 and ran for 100 days. Plants were watered with tap water to field capacity every day for the first 20 days and then three times a week thereafter. Pots were rotated every 14 days within the greenhouse to account for environmental variation.

Response to the treatments was evaluated by comparing above and belowground biomass, number of inflorescences per plant, number of tillers per plant for grasses, number of stems per plant for *A. repens*, cumulative stem height for *A. repens*, and tissue Zn and Fe concentrations, for each species. Aboveground biomass was harvested at the base of the root crown at the completion of the experiment. Soil was removed from belowground biomass by rinsing with tap water. Above and belowground biomass were oven dried for 48 h at 60 °C before weighing. All aboveground material from three randomly selected plants from each treatment/species combination grown in high-Zn soils, and three from both the mixed treatment and the low-Zn control were analyzed for Zn and Fe content (USUAL 2005). Prior to harvesting biomass, tiller number for grasses, and stem number and stem height for *A. repens* were recorded, along with the number of inflorescences for all species.

Statistical analysis

All data were analyzed by analysis of variance in PROC MIXED (SAS 1999) with a completely randomized factorial design. For both experiments, the three factors were species, soil type and treatment. Mixed soil treatments were analyzed separately, because they did not fit into the factorial design (by virtue of being a combination of the two soil types) and were compared only to the two controls for both soils. Variables were only analyzed within each species, with the following exceptions: total biomass, total tillers, and total inflorescences were compared between *P. spicata* and *P. juncea* because of the similarity in growth forms and photosynthetic pathways, and tissue Zn was analyzed across species to compare metal uptake. As analysis was not performed on plants grown in low-Zn soil with treatments, the soil factor was dropped from the factorial design and the low-Zn control and the mixed treatment were both added as additional treatments. The same approach was taken for the soil analysis, except that the species factor was also dropped. For all analyses, model significance was set at $P = 0.10$ (Cowles and Davis 1982).

Mean separation for all variables, except soil Zn and Fe, was performed using least squares differences (LSD) with an alpha value of 0.05. For soil Zn and Fe, all comparisons were used, which required a Tukey adjustment. To account for the conservative nature of the Tukey adjustment, the alpha value was set at 0.10 (Zar 1984). Data transformations were performed where necessary to meet the assumptions of normal data distribution and equal variance required for analysis of variance. Non-transformed data are presented for all results.

Results

Plant performance differed only slightly between the high and low Zn soils based on measured response variables. There were no differences in germination rates for any species (mean germination rates and standard error for each species were: *P. spicata*: 91 ± 0.01%; *P. juncea*: 72 ± 0.01%; *S. airoides* 34 ± 0.01%; *A. repens*: 53 ± 0.02%) and no differences in total biomass (*P. spicata*: 3.8 ± 0.23 g; *P. juncea*: 5.9 ± 0.23 g), or tiller number (*P. spicata*: 15.6 ± 0.87; *P. juncea*: 16.5 ± 0.87), for *P. spicata* or *P. juncea*. There were also no differences in stem number (0.7 ± 0.10), cumulative stem height (27.8 ± 4.1 cm) and inflorescence number (1.6 ± 0.30) for *A. repens*. Both *P. spicata* and *P. juncea* produced too few inflorescences to analyze. However, there were significant (Table 2) increases in total biomass for both *S. airoides* and *A. repens*, when grown in high-Zn soil (Table 3). *S. airoides* also had significant increases in tiller number and inflorescence number in the high-Zn soil.

Zn uptake increased between high-Zn and low-Zn soils, when comparing controls, and was significant for *P. spicata* and *S. airoides* (Fig. 1). The mixing of the two soils also tended to increase Zn uptake, compared to both high and low Zn soil controls for all species, and was significantly greater than both the low and high Zn controls for *P. juncea*, but only significantly greater than the low-Zn control for *P. spicata* and S. *airoides*. As P addition increased, Zn uptake decreased for *S. airoides* and *P. juncea*, and was

Table 2 Results of analysis of variance for response variables, species and factor combinations which had significant models for bio-assay species germinated and grown in high and low Zn soils

Variable	Species	Factor	df	*F*-value	P
Total biomass	S. airoides	Soil	41	10.96	0.0020
	A. repens	Soil	41	4.88	0.0330
Tiller #	S. airoides	Soil	41	17.32	0.0002
Inflorescence #	S. airoides	Soil	41	10.2	0.0027
Tissue Zn	NA	Species × treatment	74	2.85	0.0014
Soil Zn	NA	Treatment	35	15.23	<0.0001
Soil Fe	NA	Treatment	35	2.19	0.0612

Table 3 Species and response variables where significant differences occurred between plants grown in high- and low-Zn soils; compared within species (mean ± SE, LSD $\alpha < 0.05$)

Species	Soil	Total biomass (g)	Tiller/stem	Inflorescence
S. airoides	Low-Zn	8.2 ± 0.4	24.1 ± 1.5	2.8 ± 0.5
	High-Zn	10.1 ± 0.4	32.8 ± 1.5	5.0 ± 0.5
A. repens	Low-Zn	6.5 ± 0.3	0.7 ± 0.2*	1.8 ± 0.5*
	High-Zn	7.4 ± 0.3	0.8 ± 0.2*	1.5 ± 0.5*

* Nonsignificant

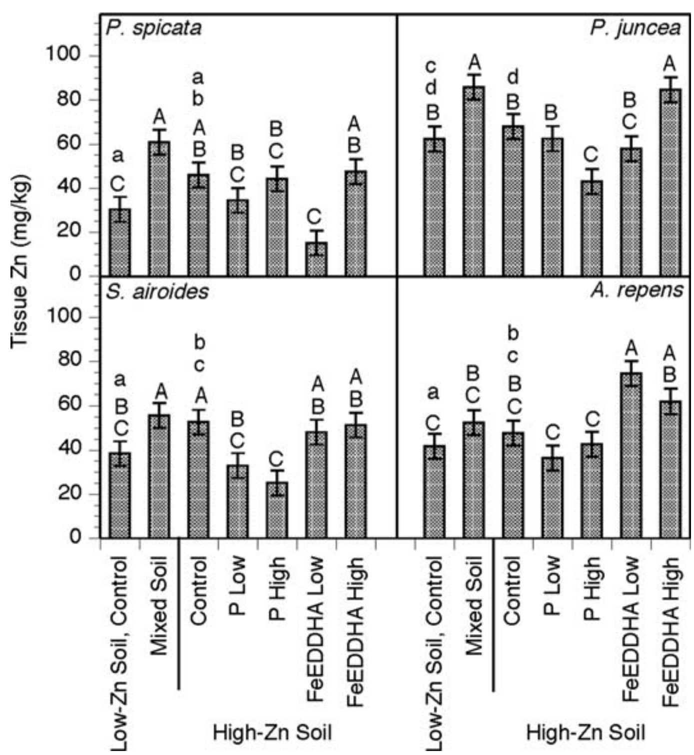

Fig. 1 Tissue Zn concentrations of plants grown in high-Zn soils with ameliorative treatments, the mixed soil treatment (high- and low-Zn soil), and the low-Zn control. Different upper-case letters indicate significant differences among means within each species. Different lower-case letters indicate significant differences between high- and low-Zn controls across species (LSD, α <0.05). Error bars represent one standard error

significantly less than the high-Zn control at the high P level for both species and also significantly less than the low P treatment for *P. juncea*. For *P. spicata* and *A. repens*, the low P treatment corresponded to a greater decrease in Zn uptake than the high P treatment, though neither was significant.

The FeEDDHA treatments resulted in both increases and decreases in Zn uptake. For *P. spicata*, there was a significant decrease associated with the low FeEDDHA treatment, while *A. repens* showed an increase. For the high FeEDDHA treatment *P. juncea* showed a significant increase in Zn uptake. Comparison of Zn uptake between species revealed that in both low- and high-Zn soils, *P. juncea* had significantly higher tissue-Zn concentrations than all other species, when control treatments were considered. In high-Zn soil, *S. airoides* had the second highest Zn concentration, followed by *A. repens* and *P. spicata*, although the differences were not significant. In low-Zn soil, *A. repens* had the second highest Zn concentration, followed by *S. airoides* and *P. spicata*, though the differences again were not significant.

Soil analysis revealed that ameliorative treatments applied to the soil in the germination experiment generally did not affect the availability of Zn in the high-Zn soil (Fig. 2). The exception was the addition of $FeSO_4$ at the high level, which significantly increased the availability of Zn compared to the controls. There was a decrease in Zn availability as Fe-oxide content increased; however, it was not significantly lower than the high-Zn control, nor did it approach the significantly lower Zn level of the low-Zn soil. Fe availability did not differ significantly among any of the treatments in the high-Zn soil, nor was it significantly different between the low and high Zn controls.

Discussion

Based on this study, there is no evidence for an inhibitory effect on any of the bioassay species from the elevated levels of bioavailable Zn associated with infestations of *A. repens*. Paschke et al. (2000) established tissue Zn toxicity thresholds for five grasses native to North America at 84–222 mg kg^{-1}. Tissue Zn levels during plant development only barely approached the lower range of these toxicity values. Additionally, there were no negative effects of elevated soil Zn on seed germination. Based on findings from associated germination studies, seeds had to be exposed to 100 mg L^{-1} of Zn in solution to cause a reduction of 10% in germination for the species used in this study (Morris 2005). Available literature regarding Zn levels in soils collected from infestations of *A. repens* indicate that the highest bioavailable Zn levels observed are generally much lower than the soils used in this study (Bottoms 2001; Tyrer 2005), which may have had overall increased Zn levels caused by nearby industrial and urban development (Garcia and Millan 1998).

These findings reflect results found in other recent research where germination and seedling development of forbs and shrubs also did not differ between soils collected from within, and adjacent to, infestations of *A. repens* (Tyrer 2005). However, the results of both of these studies are contrary to earlier work by Fletcher and Renney (1963), where soils collected from within infestations of *A. repens* did negatively impact growth and development of *Lycopersicon esculentum* Mill. (tomato) and *Hordeum vulgare* L. (barley) plants. This discrepancy may be due to differences in bioassay plants and differences in the handling of the soils. In both cases where no differences were found, native species were used as bioassays and soils were stored at least 20 days before use. The storage of soils may allow the breakdown of organic compounds, which may have been responsible for the effect found by Fletcher and Renney (1963). Additionally, agronomic species may be more susceptible to impacts from either organic compounds or elevated Zn levels.

The improvements in plant performance in the high-Zn soil for *S. airoides* and *A. repens* found in this study are most likely due to the increases in macronutrient (N and P) availability also associated with the high-Zn soil. It is possible that, if there had been a negative effect from the elevation of bioavailable Zn, the increase in macronutrients would compensate for it. The ability of *A. repens* to perform better in its own soil than in

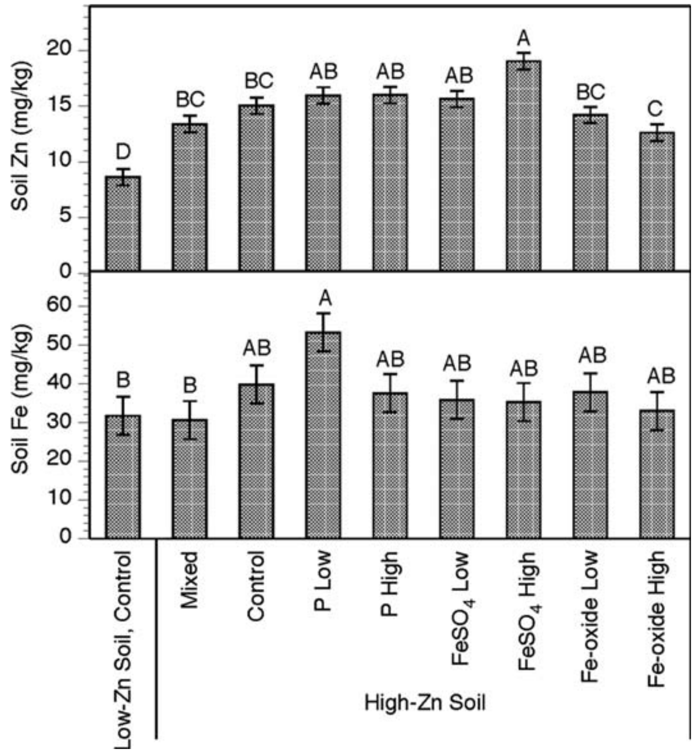

Fig. 2 Bioavailable Zn and Fe in high-Zn soils with ameliorative treatments, and in the mixed treatment and low-Zn control at the end of the 50-day germination experiment. Treatments in low-Zn soils, as well as soils from the greenhouse study were not analyzed because it was assumed that they would react similarly to treatments relative to their control. Different letters indicate significant differences between means (LSD, α <0.05). Error bars represent one standard error

a soil that has not been previously occupied by *A. repens* may represent another type of positive feedback, not associated with Zn phytoenrichment. Wilson and Agnew (1992) describe a feedback response where a plant increases macronutrient availability over time, which it is able to capitalize on more than its neighbors, resulting in its improved success. Klironomos (2002) also provides evidence that feedback with soil microorganisms can explain the success of some invasive species. In particular, invasive species benefited from the presence of mycorrhizal fungi collected from soils they had previously occupied. Further studies would be needed to determine whether *A. repens* was benefiting more overall than native species, as *S. airoides* also benefited from the soils previously occupied by *A. repens*.

In terms of tissue Zn content, it should be noted that *P. juncea* had significantly higher tissue Zn concentrations than all of the other species, and tissue Zn concentrations for *A. repens* were not significantly higher than either of the native grasses. Levels in *A. repens* were slightly higher in this study than values reported by Bottoms (2001). Results for perennial grasses in this study were much higher than previously reported, and were either statistically similar to the values for *A. repens*, or in the case of *P. juncea*, higher. This discrepancy between the two studies was likely caused by the differences in soil volume available for exploitation by plants in the two studies, combined with differences in root architecture between the grasses and *A. repens*. Taproots of *A. repens* are known to penetrate to depths of 6 m (Selleck 1964) and, as *A. repens* is known to accumulate Zn and concentrate it at the surface, it can be assumed that deeper roots and large volumes of soil are involved in the process of Zn accumulation. Perennial grasses, on the other hand, are known to concentrate fibrous roots at the surface in order to capture pulses of moisture and nutrients (Caspar and Jackson 1997) and would therefore be less restricted in their Zn uptake by confinement in a pot than *A. repens*.

Another important factor in the accumulation of Zn in plant tissues is the age of the plant. Since the plants used in this study were only 100 days old, they would be expected to have lower Zn levels than older plants. In addition, Zn and other heavy metals may have lower concentrations in developing seedlings because of dilution effects from high relative growth rates (Lawlor 1991). Mature plants with lower rates of growth, that have been exposed to elevated soil Zn levels longer will likely accumulate higher concentrations and be more susceptible to effects from elemental allelopathy.

The treatments applied to soils to alleviate Zn stress had both predictable and surprising responses in relation to soil Zn and Fe. The lack of change associated with the P treatments is not surprising since, although it can be highly reactive with Zn, forming insoluble precipitates, P can also effectively reduce Zn activity at the tissue level, without altering the availability of Zn in the soils (Loneragan and Webb 1993). The increase in Zn availability associated with the high $FeSO_4$ treatment may be attributed to the replacement of Zn by Fe on exchange sites. The lack of increase in bioavailable Fe associated with the $FeSO_4$ treatments may be caused by the rapid oxidation of Fe and precipitation as insoluble Fe oxides (Lindsay 1979). Thus, there would be no effect upon Fe availability since measurements were made at the end of the study where the form of Fe consisted of insoluble oxides for both the $FeSO_4$ treated and untreated soils, which would have resulted in the same DTPA Fe extractable values. This increase in Zn availability with no corresponding increase in Fe availability negates its usefulness as an ameliorative agent.

Many of the treatments for ameliorating Zn stress in this study were chosen because of their applicability to management actions. However, as there was no evidence of a negative effect from elevated soil Zn in this study, application of these treatments is not warranted as an aid in the restoration of desirable grasses. In particular, the reduction of Zn concentrations in topsoils through plowing or other mixing treatments are not justified, especially for wildlands. The disturbance to soil may encourage colonization by weedy species (Sheley et al. 1999), and appears to actually increase Zn uptake. This supports the need for complete investigation of the effects from elemental allelopathy by following the guidelines suggested by Fuerst and Putnam (1983) in order to avoid taking unnecessary and potentially destructive or costly management actions.

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